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Dynamic Mathematical Model To Predict Microbial Growth and Inactivation during Food Processing

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Many sigmoidal functions to describe a bacterial growth curve as an explicit function of time have been reported in the literature. Furthermore, several expressions have been proposed to model the influence of temperature on the main characteristics of this growth curve: maximum specific growth rate, lag time, and asymptotic level. However, as the predictive value of such explicit models is most often guaranteed only at a constant temperature within the temperature range of microbial growth, they are less appropriate in optimization studies of a whole production and distribution chain. In this paper a dynamic mathematical model—a first-order differential equation—has been derived, describing the bacterial population as a function of both time and temperature. Furthermore, the inactivation of the population at temperatures above the maximum temperature for growth has been incorporated. In the special case of a constant temperature, the solution coincides exactly with the corresponding Gompertz model, which has been validated in several recent reports. However, the main advantage of this dynamic model is its ability to deal with time-varying temperatures, over the whole temperature range of growth and inactivation. As such, it is an essential building block in (time-saving) simulation studies to design, e.g., optimal temperature-time profiles with respect to microbial safety of a production and distribution chain of chilled foods.

Nowadays, there is an increased consumer interest in chilled prepared food products. However, the shelf life of this kind of food is usually limited because of spoilage by common microorganisms, such as *Pseudomonas* and *Lactobacillus* spp., and the increased risk for food pathogens.

In predicting the shelf life, the use of mathematical models is gaining more and more attention to increase insight in the different subprocesses and their interactions. For process design and optimization, simulation studies can be very useful to reduce the number of expensive and time-consuming experiments.

It is well known that temperature, pH, and water activity are the main factors influencing the microbial stability of these foods. Unlike the pH and the water activity, the temperature may vary extensively throughout the complete production and distribution chain. It follows that a more general modeling approach, in which mathematical models for heat transfer, microbial load, and other process variables (e.g., sensory quality) at any location in the food (with well-known pH and water activity) are combined and interconnected, is required.

A model-based approach was pioneered in the sterilization of canned foods, for which an optimization methodology already exists (e.g., see the work of Saguy and Karel [7], Nadkarni and Hatton [4], and Teixeira and Shoemaker [9]). In sterilization processes a drastic thermal treatment results in a complete inactivation of vegetative cells and a considerable reduction of spores of clostridia at the expense of the sensory quality. The kinetics of thermal inactivation are well documented (e.g., see the work of Stumbo [8]). Under normal storage conditions and in the absence of postcontamination, usually no further microbial growth occurs.

However, the construction of a dynamic model for the microbial load in chilled prepared foods presents some specific problems. The objective of the thermal treatment is to obtain an optimal textural and sensory quality, while the reduction of pathogenic and spoiling vegetative cells is a derived benefit. The latter is less intensive than in canning, and thermoresistent microorganisms (or their spores) may survive. During the subsequent stages of chilling, transport, storage, and distribution, the temperature within the product can fluctuate between 0 and 30°C, and the surviving microorganisms may start growing. To our knowledge, there have been up to now no mathematical models available which are capable of predicting microbial growth in foods under dynamically changing temperature conditions.

In this paper, we focus on models describing the bacterial population evolution with respect to time and temperature. The possible influence of pH and water activity is not considered.

Recently, Zwietering et al. (10, 11) compared several explicit models for bacterial growth, either reported in the literature or newly developed, using a lot of experimental data. Different models were compared statistically, as well as with respect to their ease of use. They reparameterized the models in order to obtain biologically significant parameters, such as the maximum specific growth rate (μ_m), the lag time (λ), and the asymptotic level (A) of the growth curve. They concluded that in almost all the cases, the three-parameter Gompertz model (2) can be regarded as the best one in modeling the logarithm of the relative population size [$y = \ln (N/N_0)$] (N is the number of microorganisms per unit of volume) as a function of time (t). Furthermore, they

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selected the models to describe the three main growth characteristics, μ_m, λ, and A, as functions of temperature. Unfortunately, the predictive value of these static models can be guaranteed only at a constant temperature within the temperature range of microbial growth. In practice, however, the food product may be subjected to wide temperature variations. This hampers the application of these models in process design and control.

The main features of the dynamic model presented in this paper are as follows. First of all, both microbial growth and inactivation are described with a single expression. A possible transition zone between growth and inactivation can be simulated easily. Second, as the model is in the form of a first-order differential equation instead of an explicit function, it can deal with time-varying temperatures in a consistent way. This will be explained later on. As an additional feature, the previous history of the product under consideration can be taken into account in a natural way. Third, at a constant temperature within the range of growth, it reduces to the Gompertz model as proposed by Zwietering et al. (10, 11), which describes reported experimental data very well. Finally, the model has the required mathematical properties to make modern optimization techniques easily applicable. As a result, this model can be one of the building blocks of a complete simulation model, useful in process design and optimization.

We wish to emphasize from the outset that the principal aim of this paper is to introduce more-advanced concepts of mathematical modeling into the field of predictive microbiology. More specifically, the model developed here is a first step towards a realistic and consistent description of the evolution of a microbial population under time-varying temperature conditions. The experimental validation of the model under such temperature conditions is the subject of an ongoing project within our research group.

THEORY

Modeling the bacterial growth curve as a function of time. Following the work of Zwietering et al. (11), we define the growth curve as the logarithm of the relative population size [$y = \ln (N/N_0)$] as a function of time (t). Using extensive experimental data, Zwietering et al. concluded that the Gompertz model (chosen from a large number of explicit models either proposed in the literature or newly developed) can be regarded as the best one. They reparameterized the original Gompertz model (2)—with parameters a, b, and c—in order to obtain biologically meaningful parameters, such as the maximum specific growth rate μ_m, the lag time λ, and the asymptotic value A.

The original Gompertz equation (2) is as follows:

$$y = a \exp[-\exp(b - ct)] \tag{1}$$

The modified Gompertz equation [where $e = \exp(1)$] (11) is as follows:

$$y = A \exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\} \tag{2}$$

The conversion formulas are as follows:

$$a = A \tag{3}$$

$$b = \frac{\mu_m e}{A}\lambda + 1 \tag{4}$$

$$c = \frac{\mu_m e}{A} \tag{5}$$

Modeling μ_m, λ, and A as functions of temperature. To cope with the influence of the temperature (T), Zwietering et al. (10) selected for μ_m, λ, and A the most appropriate model, either existing or newly developed. As a result, modified versions of the square root model of Ratkowsky et al. (6) have been proposed for both μ_m and A. The square root model of Ratkowsky et al. (6) is as follows: $\sqrt{\mu_m} = b_1(T - T_{min})$, where T_{min} is the theoretical minimum temperature for growth. Ratkowsky et al. (5) expanded their model to describe the growth rate around the optimum and the maximum temperatures. The expanded square root model of Ratkowsky et al. (5) is as follows:

$$\sqrt{\mu_m} = b_2 (T - T_{min})\{1 - \exp[c_2(T - T_{max})]\} \tag{6}$$

where T_{min} is the minimum temperature at which growth is observed and T_{max} is the theoretical maximum temperature for growth. To avoid positive values for μ_m at temperatures $>T_{max}$, Zwietering et al. (10) proposed the following modified Ratkowsky model:

$$\mu_m = b_3^2 (T - T_{min})^2\{1 - \exp[c_3(T - T_{max})]\} \tag{7}$$

Zwietering et al. (10) found that from a statistical point of view, the last two models describe the growth rate data sufficiently. They preferred model equation 7, as it had the lowest residual sum of squared errors. However, the difference with the expanded Ratkowsky model is almost negligible.

For the asymptotic level A of the growth curve, the following expression based on the model of Ratkowsky et al. has been selected (10):

$$A = b_4\{1 - \exp[c_4(T - T_{A,max})]\} \tag{8}$$

where b_4 is the final level at low temperatures and $T_{A,max}$ is the maximum temperature at which growth is observed. Assuming that the final population N_∞ is independent of the inoculum level, b_4 depends on the inoculum level.

For the description of the lag time λ as a function of temperature, Zwietering et al. (10) selected the following hyperbolic model:

$$\ln (\lambda) = \frac{p}{T - q} \tag{9}$$

The parameters obtained by Zwietering et al. (10) for the models shown by equations 6 to 9 are summarized in Table 1.

Limitations of the current explicit model. As already mentioned, the predictive value of the model composed of equations 2 and 7 to 9 is guaranteed only at a constant temperature within the temperature range of bacterial growth. Zwietering et al. (10) reported an excellent agreement with measured values. However, this model cannot be used under time-varying temperature conditions. Consider, e.g., the following temperature profile. From $t = 0$ h to $t = 20$ h, T equals 10°C; at $t = 20$ h, the temperature jumps to 30°C. In Fig. 1, the output of the explicit model of Zwietering et al. (10) is compared with the result of the dynamic model we shall present in the next sections. Although the solutions coincide during the first phase, the model of Zwietering et al. cannot handle the temperature shock in a consistent way: at the moment of the temperature shock, the population makes also a (discontinuous) jump which is, of course, impossible.

TABLE 1. Parameters reported by Zwietering et al. (10) for the models shown by equations 6 to 9

Model (equation no.) and parameter	Estimate
Expanded Ratkowsky model (6)	
b_2	0.0377
T_{min}	2.82
c_2	0.250
T_{max}	44.9
Modified Ratkowsky model (7)	
b_3	0.0410
T_{min}	3.99
c_3	0.161
T_{max}	43.1
Asymptote A (8)	
b_4	21.58 − ln (N_0)
c_4	1.25
$T_{A,max}$	43.1
Lag time λ (9)	
p	23.9
q	2.28

On the other hand, the prediction of the dynamic model coincides with what might be expected intuitively: from the temperature shock on, the population starts growing faster but remains continuous. In other words, the new dynamic model can take into account the previous history of the product at hand.

Observe that both model equations 7 and 8 predict unrealistic negative values for, respectively, μ_m and A at temperatures above the maximum temperature for growth. At temperatures below the minimum temperature for growth, model equation 7 predicts unrealistic positive values for μ_m.

In addition, Zwietering et al. (10, 11) use the Gompertz model as an empirical model for describing the logarithm of the relative population size [$y = \ln (N/N_0)$] with time, while the original Gompertz model (2) was developed to describe the evolution of the absolute population size N. Their approach can be justified by the excellent fitting results of experimental data. However, there is some inconsistency from the mathematical point of view. At $t = 0$, we have $N(t = 0) = N_0$, so $y(0) = 0$. On the other hand, from model equation 1 or 2 it is readily seen that $y \to 0$ for $t \to -\infty$ only. At $t = 0$, we have

$$y(0) = a \exp[-\exp(b)] = A \exp\left[-\exp\left(\frac{\mu_m e}{A}\lambda + 1\right)\right] \quad (10)$$

which is not equal to zero. For the values of the parameters estimated by Zwietering et al. (10), $y(0)$ only approaches zero. This has some consequences in developing a consistent dynamic model, as we shall see further on.

Design requirements for a dynamic model. (i) The dynamic model should be able to deal with time-varying temperature profiles in a consistent way, over the whole biokinetic temperature range of growth and inactivation. All variables must take on physically acceptable values under all conditions. (ii) The model should be able to simulate a transition (whether smooth or not) between growth and inactivation, using as few additional parameters as possible (in order to avoid unnecessary complications in parameter estimation studies). (iii) The previous history of the food product under consideration should be taken into account. At the beginning of a growth phase after a phase of inactivation, the reference population level N_0 should be modified accordingly. This will be explained later on. (iv) The model should reduce to the validated existing explicit model, in the special case of a constant temperature within the range of growth. (v) The model should meet some mathematical requirements, such as differentiability for all values, in order to make some nonlinear parameter estimation algorithms and modern optimization techniques easily applicable.

A dynamic mathematical model. (i) A dynamic model for growth. From the mathematical point of view, the main reason that the model of Zwietering et al. (10) fails under time-varying temperature conditions (Fig. 1) is that this

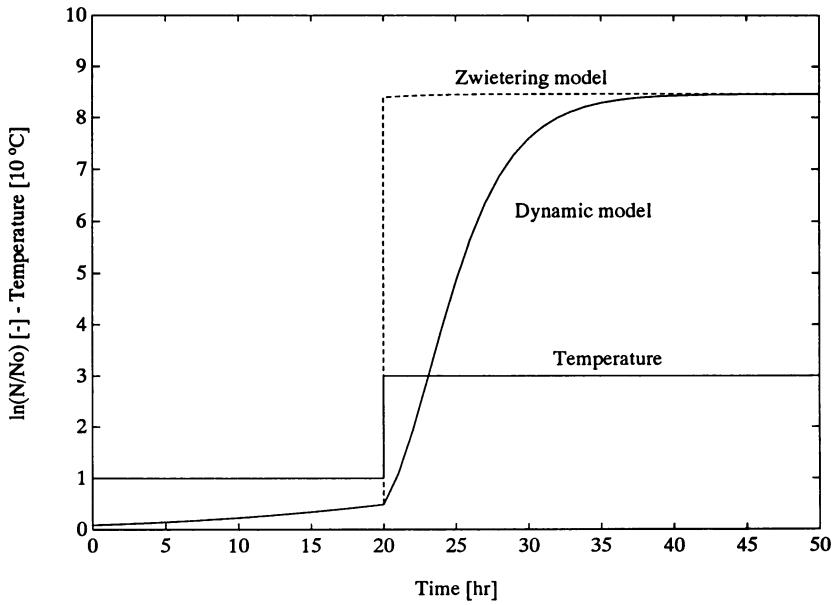

FIG. 1. Prediction of the explicit model of Zwietering et al. (10) versus the dynamic model for a step in the temperature profile.

model must be considered the explicit solution of a more general (dynamic) differential equation in the special case of a constant temperature. The underlying differential equation for model equation 1 (or 2) can be easily obtained by differentiating y with respect to t. We have

$$\frac{dy}{dt} = a \exp[-\exp(b - ct)][-\exp(b - ct)](-c)$$

$$\frac{dy}{dt} = cy \ln\left(\frac{a}{y}\right) \quad (11)$$

Unfortunately, parameter b and thus the influence of the lag time λ due to equation 4 have disappeared completely from this dynamic model. Furthermore an initial condition, $y(t = 0) = 0$, as required by the definition of y itself, causes a numerical problem in the right side of equation 11, although the limit for $y \rightarrow 0$ can be proven to be zero.

Both problems can be circumvented by using the initial condition as given by equation 10. By using conversion formulas 3 to 5 and model equations 7 to 9, this dynamic model becomes function of T also. By doing so, we also guarantee the complete equivalence of dynamic model 11 with explicit model 1 in the case of a constant temperature.

It is now easily seen that the jump in the temperature profile of Fig. 1 causes a discontinuity in the values of μ_m and λ and, as a consequence, in the value of y modeled by equation 1. However, by using dynamic model 11, there is a jump only in the derivative of y, y itself remaining continuous. The influence of the temperature shock is smoothed through the integration process, which results in a more realistic time profile $y(t)$. This is one of the main advantages of the new dynamic model.

In order to handle the third model design requirement on N_0 in an easy way, we define the following variable:

$$n = \ln(N) \quad (12)$$

Note that $dy/dt = dn/dt$. With $n_0 = \ln(N_0)$, we obtain the following dynamic model for n:

$$\frac{dn}{dt} = c(n - n_0) \ln\left(\frac{a}{n - n_0}\right) \quad (13)$$

$$n(t = 0) = n_0 + a \exp[-\exp(b)] \quad (14)$$

The initial condition 14 follows immediately from equation 10.

(ii) A dynamic model including inactivation. As a first step, we make the model for growth consistent outside the interval $[T_{min}, T_{max}]$. Kohler et al. (3) proposed the following modification of the expanded Ratkowsky model (equation 6):

$$\mu_m = 0 \text{ if } T < T_{min}$$

$$\mu_m = b_2^2 (T - T_{min})^2 \{1 - \exp[c_2(T - T_{max})]\}^2 \quad (15)$$

$$\text{if } T_{min} \leq T \leq T_{max}$$

$$\mu_m = 0 \text{ if } T > T_{max}$$

Because of conversion formula 5, c equals 0 outside the interval $[T_{min}, T_{max}]$. As a result, y remains then constant (equation 11), which is the desired behavior. Note that this model is both continuous and differentiable for all values of T, which makes it attractive from the mathematical point of view also.

Furthermore, the prediction by model equation 8 of negative asymptote values A for $T > T_{A,max}$ can be circumvented as follows. It is clear that around the transition temperature $T_{A,max}$, there are two phenomena occurring at the same time: microbial growth and high-temperature inactivation. A realistic model should exhibit some transition point (or even a transition zone), where the net effect of both reactions is almost zero: growth and inactivation compensate each other almost completely. The most consistent and elegant solution is to model both competing processes independently of each other. As a result, we propose the asymptote A to be independent of the temperature T:

$$A = b_4 = A_0 - \ln(N_0) = A_0 - n_0 \quad (16)$$

In the food engineering literature, it is common practice to model the high-temperature inactivation rate constant k (per hour) with an Arrhenius-type model proposed by Bigelow (1):

$$k(T) = 60 \left(\frac{2.303}{D_{ref}}\right) \exp\left[\frac{2.303}{z}(T - T_{ref})\right] \quad (17)$$

where D_{ref} is the time (minutes) required to reduce the bacterial concentration by a factor of 10, at the reference temperature T_{ref}, while z is the increase in temperature necessary to reduce this time requirement by the same factor. In order to model a possible transition zone between growth and inactivation in which y remains constant, we propose the following modification of the model of Bigelow:

$$k_{mod}(T) = f_{trans}(T)k(T) \quad (18)$$

$$f_{trans}(T) = \exp\{-\exp[\alpha(T_{trans} - T)]\} \quad (19)$$

The temperature T_{trans} determines the length of the transition zone ($T_{trans} - T_{max}$), while the parameter α determines the transition velocity around T_{trans} from growth to inactivation. Both parameters depend on the bacterial population under consideration. The function $f_{trans}(T)$ is plotted in Fig. 2 for $T_{trans} = 50°C$ and some values of α. It can be seen that for $\alpha \rightarrow \infty$, $f_{trans}(T)$ approaches a unit step function at $T = T_{trans}$. In the special case of $T_{trans} = T_{max}$, there is no transition zone at al.

The overall specific growth rate μ (per hour) is defined as follows:

$$\frac{dN}{dt} = \mu N \quad (20)$$

By using definition 12 it follows that

$$\frac{dn}{dt} = \mu \quad (21)$$

Observe that the overall rate μ includes both growth and inactivation. We obtain the following dynamic model:

$$\frac{dn}{dt} = [1 - f_{trans}(T)]c(n - n_0) \ln\left(\frac{A_0 - n_0}{n - n_0}\right) - f_{trans}(T)k(T) \quad (22)$$

$$n(t = 0) = \ln(N_0) + a \exp[-\exp(b)] \quad (23)$$

Note that in this model equation, the sum of the weighting factors for growth and inactivation is equal to 1, thus assuring a smooth transition if α is low.

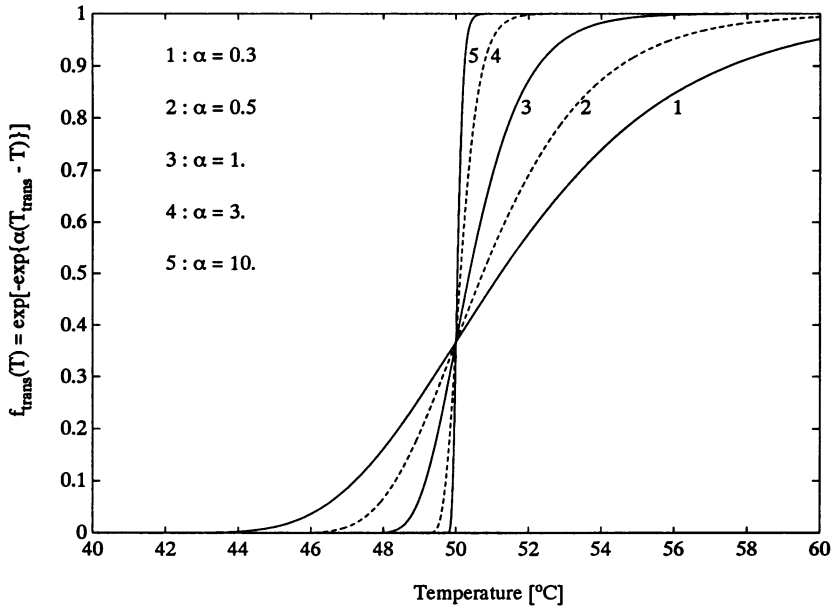

FIG. 2. Value of the transition function $f_{trans}(T)$ for some values of the parameter α and T_{trans} = 50°C.

The third model design requirement can be explained as follows. It can be expected that at the beginning of a new growth phase after an inactivation phase, the organism has to adapt again during some time to the lower-temperature conditions. This results in a new lag time. In order to make consistent predictions with model equation 22, the value of $n_0 = \ln(N_0)$—which plays the role of a reference level—must be changed accordingly. It follows that n_0 must be variable with time t. By doing so, we take into account the previous history of the food product in a natural way. The desired behavior can be modeled by the following dynamic model for n_0:

$$\frac{dn_0}{dt} = \gamma f_{zero}(T)\{n - n_0 - a \exp[-\exp(b)]\} \quad (24)$$

$$n_0(t = 0) = \ln(N_0) \quad (25)$$

$$f_{zero}(T) = \exp\{-\exp[\beta(T_{zero} - T)]\} \quad (26)$$

This can be explained as follows. $f_{zero}(T)$ is a switching function similar to $f_{trans}(T)$. T_{zero} is the temperature at which the overall specific growth rate μ (equations 21 and 22) changes sign and must be determined numerically. From these equations it can be seen that in general T_{zero} depends on the (logarithmic) population size n. In Fig. 3 are shown some curves for T_{max} = 44.9°C and some values of T_{trans} and α. If $T_{trans} = T_{max}$ (upper plot), i.e., if there is no transition zone, then T_{zero} approximates T_{max} only for very large values of α. However, in the generic case $T_{trans} > T_{max}$, the lower plot indicates that for all values of n, T_{zero} almost equals T_{max} from α = 2 on and needs not to be determined numerically anymore.

β is set equal to a very large positive value (e.g., β = 100), so that $f_{zero}(T)$ approaches a unit step function at $T = T_{zero}$. Note that, in contrast with parameter α in equation 19, β does not depend on the microbial population under consideration. During a growth phase ($T < T_{zero}$) $f_{zero}(T)$ equals 0 (at least from the numerical point of view), so n_0 remains constant. During an inactivation phase ($T > T_{zero}$) $f_{zero}(T)$ equals 1, so n_0 moves towards $\{n - a \exp[-\exp(b)]\}$ with a velocity determined by the factor γ (which is at the disposal of the user) and then tracks this value. At the beginning of a subsequent growth phase, the difference $(n - n_0)$—needed in model equation 22—is at its desired value $a \exp[-\exp(b)]$ with an accuracy determined by γ. This is equivalent with the initial condition 14 for the dynamic model for growth only. Note that $a \exp[-\exp(b)]$ contains the lag time λ in an implicit way through equation 4. From the mathematical point of view, $n - n_0$ never becomes negative, thus making the right side of model equation 22 always computable. In summary, the complete dynamic model is as follows: equations 22 to 25; for growth, equations 4, 5, 9, 15, and 16; for inactivation, equation 17; and for transition, equations 19 and 26.

RESULTS AND DISCUSSION

In this section we check whether the design requirements for a dynamic model as stated above are sufficiently fulfilled by the proposed model. During all simulations mentioned, the following parameter set was used: for growth, A_0 = 21.58 and γ = 100; for inactivation (taken from the work of Stumbo [8]), D_{ref} = 1.00, z = 10, and T_{ref} = 65.6; and for transition, T_{trans} = 44.9, α = 1, and β = 100. For the other parameters concerning growth, reference is made to Table 1. Observe that in the model of Kohler et al. (equation 15) (3) the parameters of the expanded square root model (equation 6) can be used immediately. The parameters given in Table 1 are from *Lactobacillus plantarum* (10). Since no parameters describing high-temperature inactivation are available, the parameters for a *Lactobacillus* sp. (reported by Stumbo [8]) are given above. The value of the inoculum level N_0 is set equal to 5×10^5.

First of all, a transition zone between growth and inactivation can be easily simulated by using the transition function $f_{trans}(T)$ (equation 19). The overall specific growth rate μ as a function of temperature is given by the right side of

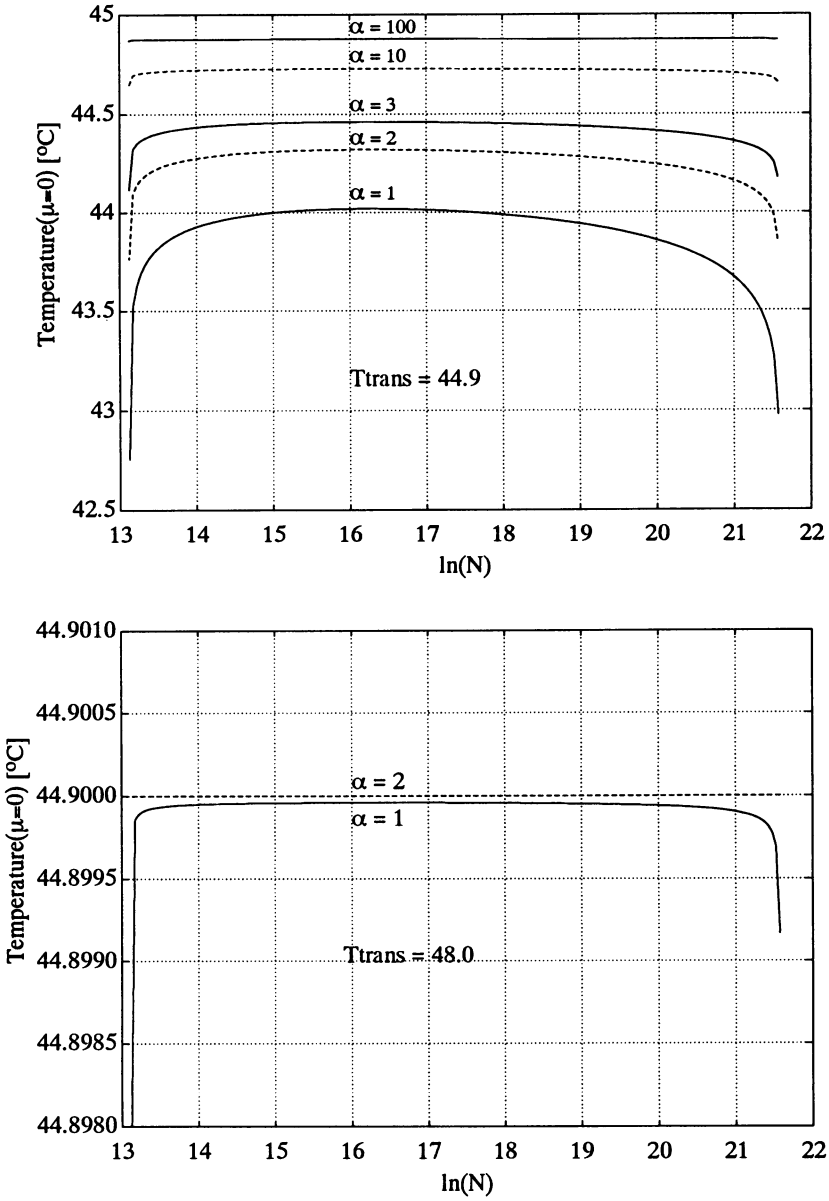

FIG. 3. T_{zero} as a function of n = ln (N) for some values of T_{trans} and α and T_{max} = 44.9°C.

equation 22. In Fig. 4, some of the possibilities are illustrated. In this plot, n is chosen such that max(μ) = μ_m. The first curve, obtained by letting $T_{trans} = T_{max}$ and $\alpha = 1$, represents a microorganism with no transition zone at all. The very smooth behavior around the transition point T_{max} is obtained with a low value of α. The second curve exhibits a transition zone with zero overall specific growth rate between T_{max} and T_{trans}. The low value of α results again in a smooth behavior at temperatures near T_{trans}. Observe that both parameters T_{trans} and α depend on the microbial population under consideration: α is a curve-fitting parameter, and T_{trans} has a clear physical interpretation. Obviously, the shape of the transition zone in the overall specific growth rate is rather sensitive to the value of T_{trans}. This indicates that T_{trans} can be determined uniquely if sufficient experimental data around T_{trans} are available.

Differentiability for all times and temperatures is guaranteed by the construction of the model itself. Note that we proposed a smooth transition function $f_{zero}(T)$ (equation 26) instead of a simple unit step function. As a result, derivation with respect to temperature can be done analytically for all values of T. As such, modern optimization techniques (such as optimal control theory) become easily applicable.

Furthermore, the model proposed can deal with time-varying temperature profiles in a consistent way, over the whole temperature range of growth and inactivation. In Fig. 5, the predicted response of a microbial population to a hypothetic time-temperature profile is shown [N = exp(n) is the absolute population size per unit of volume]. This plot sufficiently illustrates the capabilities of the model. After a phase of inactivation, the value of the reference level N_0 = exp(n_0) is indeed at the value required in the subsequent growth phase. In the second growth phase, there is an

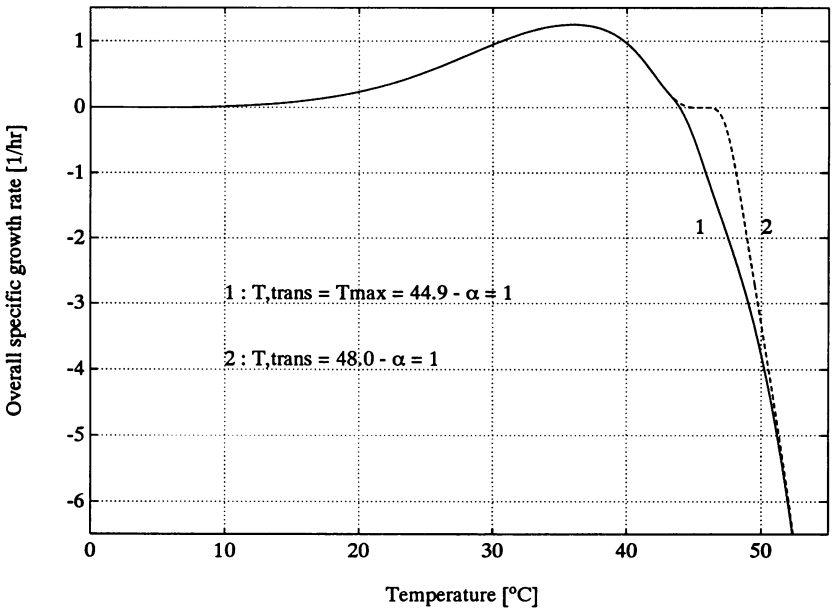

FIG. 4. Overall specific growth rate as a function of temperature.

additional lag around t = 50 h, due to the very low temperatures, as could be expected.

Finally, it can be easily seen that the dynamic model reduces by construction to the experimentally validated explicit model as proposed by Zwietering et al. (10, 11) in the special case of a constant temperature within the range of microbial growth. This is also illustrated in Fig. 1 for values of t lower than 20 h and in Fig. 5 for values of t lower than 40 h.

As for the parameter sensitivity, the following observations can be made. First, all parameters concerning growth are taken from the work of Zwietering et al. (10). More details concerning the practical identifiability and the determination of confidence intervals starting from experimental data are reported there and are not repeated here. Suffice it to say that all parameters describing μ_m, λ, and A can be determined from experiments carried out at a lot of different but constant temperatures. In addition, Kohler et al. (3) demonstrated that when modeling the maximum specific growth rate μ_m with equation 15 instead of using the expanded Ratkowsky model (equation 6)—in other words, using μ_m as the dependent variable instead of $\sqrt{\mu_m}$—best-fit estimates of the parameters can easily be obtained by employing a standard implementation of the Marquardt-Levenberg algorithm. Model equation 15 is a close-to-linear model (3). This means that a least-square estimator, even

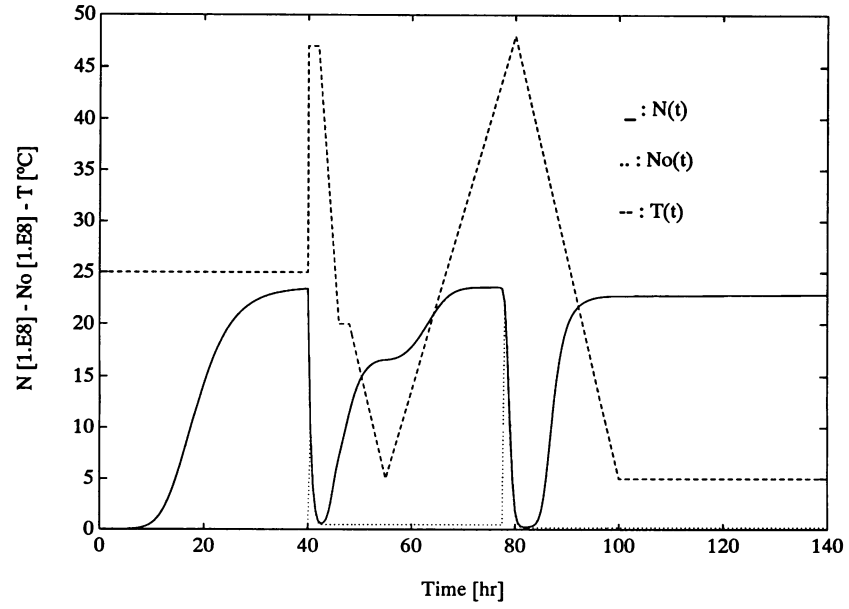

FIG. 5. Predicted response of a microbial population to a hypothetic time-temperature profile.

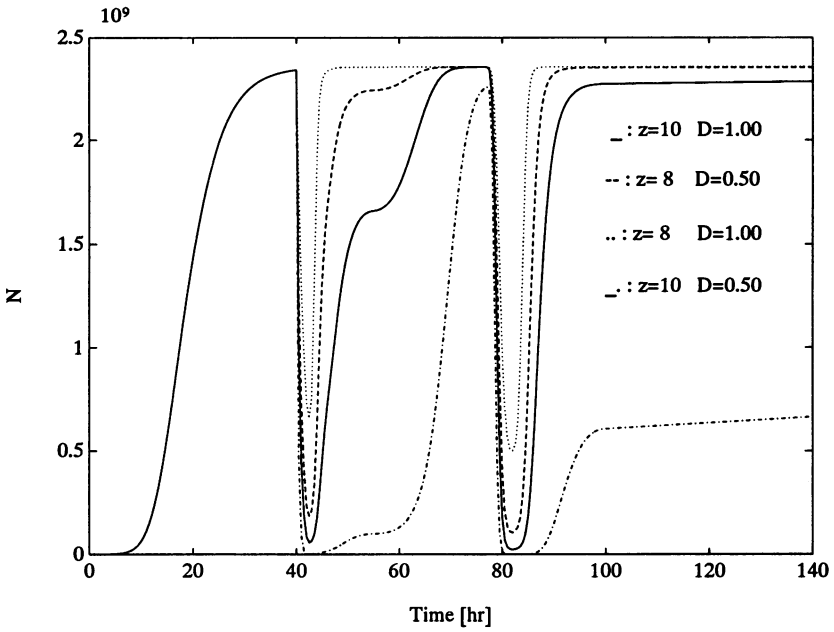

FIG. 6. Sensitivity of the predicted response of a microbial population to variations in D_{ref} and z.

when sample sizes are relatively small, comes close to being an unbiased, normally distributed, minimum-variance estimator. As a result, confidence intervals for the parameters can be readily constructed. Second, the sensitivity of the transition zone to variations in T_{trans} has been illustrated in Fig. 4. Third, the values for the parameters D_{ref} and z (describing high-temperature inactivation) are in fact the maximum values, for which Stumbo (8) reported the following range for a *Lactobacillus* sp.: D_{ref} in the interval [0.50, 1.00] and z in the interval [8, 10]. A detailed analysis of the practical identifiability of these parameters is beyond the scope of this paper. Reference is made to, e.g., the work of Stumbo (8) and the references therein. In Fig. 6, the sensitivity of the predictions by the new model towards different values for D_{ref} and z by using the same hypothetic time-temperature profile as in Fig. 5 is illustrated. Clearly, both parameters have a large influence on the evolution of the total population number N with respect to time. On the other hand, these simulation results suggest that both parameters may be determined uniquely if designing an experiment using a similar dynamic time-temperature profile.

Therefore, we suggest the following two-step procedure for the estimation of the parameters of the new dynamic model. In a first step, experiments are carried out at a lot of different but constant temperatures. This allows modeling of the growth characteristics μ_m, λ, and A as functions of temperature by using model equations 9, 15, and 16. In addition, experimental data of the specific growth rate at temperatures around T_{trans} allow for the estimation of T_{trans} and α. In a second step, experiments carried out using a dynamic time-temperature profile as illustrated in Fig. 6 can be helpful to refine the value of the parameters D_{ref} and z (taken from, e.g., literature data). As already mentioned, T_{zero} can then be calculated numerically from the right side of equation 22 if all parameters are known. Remember that the parameters β (equation 26) and γ (equation 24) are independent of the microbial population under consideration and are set at a large (positive) value.

As already mentioned in the introduction, the principal aim of this paper was to introduce more-advanced modeling concepts into the field of predictive microbiology. The new dynamic model presented establishes a general framework for describing a microbial population under time-varying conditions in a consistent way: the simulations indicate that the predicted population evolution coincides with what may be expected intuitively. We wish to emphasize again that further refinements will be certainly required after an experimental validation study. More specifically, the validity of model equations 9 and 15 to 17 under time-varying temperature conditions must be checked. It can be expected, e.g., that for the same initial population size, the lag time λ will depend on whether the growth phase follows an inactivation phase. Furthermore the possible influence of pH and water activity must be considered.

However, as these refinements are only at the level of modeling the main characteristics λ, μ_m, A, and k, the general approach presented in this work will remain valid.

Conclusions. The main contributions of this paper can be summarized as follows. We proposed a dynamic model describing a bacterial population as a function of both time and temperature, over the whole biokinetic temperature range of growth and inactivation. The main feature of this model is its ability to deal with time-varying temperature conditions: although not yet validated in practice, the model predictions look at least very meaningful and can be interpreted from a microbiological point of view. In contrast to an explicit model, this dynamic model can take into account the previous history of the food product in a natural way.

Furthermore, in the special case of a constant temperature within the range of growth, the model predictions coincide exactly with the experimentally validated explicit Gompertz model. The model can easily simulate microorganisms with a quite different behavior at temperatures around the transition from growth to inactivation, by using a simple transition function with clear parameters. Finally, the complete model has suitable mathematical properties to make advanced

nonlinear parameter estimation schemes and modern optimization techniques easily applicable.

As such, this model is an appropriate building block of a global model, useful for prediction and control of microbial growth during thermal treatment and storage of chilled foods.

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